ON THE SIGNIFICANCE OF THE POLAR SPOT IN RIPE UNFERTILIZED AND IN FERTILIZED ASCIDIAN EGGS

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Cohen and Berrill (1936) have given an interesting account of observations on eggs of *Ascidiella aspersa* and *Phallusia mamillata*. After the jelly was digested by the gastric juice of the crab *Munida*, the eggs were stained *in toto* with a vital dye (particularly Nile-blue sulfate) and subsequent events studied with special reference to changes in the form of the germ and in the appearance of the vital dye within the egg. These are described in the ripe egg, from the time of fertilization until the formation of the polar bodies, and during cleavage, gastrulation, and formation of the tadpole.

In the ripe unfertilized egg they have observed the "polar pit" ("tache polaire" of *Ascidiella*—Dalcq, 1932) though they question whether this cortical differentiation marks the position of the maturation spindle and identifies the location of the animal pole. Their doubt is not based on continuous observation of the region of the polar pit from which the dye entirely disappears before the elevation of the polar body, nor have they been able to determine directly whether the polar body is actually formed in the polar pit or not. They are influenced, however, by certain cases in which foreign particles adhere to the cortical layer of naked eggs in the vicinity of the polar pit. On measuring the distance between such a particle and the pit, and, later, between the same particle and the polar body, they found considerable variation in the distances. In two cases, drawings of which are given in their paper, the distance was increased; the same was true of "several" other eggs. In one case the distance did not change. While Cohen and Berrill consider the possibility of a displacement of the marking particle by some movement of the cortical layer, they discard such an explanation on the following grounds. During the maturation period the egg shows, grossly, only a flattening and an elongation, while the deformation during cleavage, though much greater, is unaccompanied by any change in the distance between the polar body and the adhering particle. Hence they do not believe it possible that the earlier deformations of the egg could be responsible for the displacement of the small body fixed to the cortex. They feel justified in stating, therefore: "Since Dalcq accepted the site of the polar pit as being identical with that of the polar body and

used it to orient the eggs for cutting, it follows that some doubt is thrown on the validity of his conclusions regarding the localization of presumptive germinal regions in the fertilized egg of *Ascidiella aspersa*, although, of course, not on the existence of such presumptive regions" (p. 84). They do not state what, in their opinion, the significance of the polar pit is. On the basis of experiments cited below we have good reason to believe that their conclusion is not justified.

It is unfortunate that Cohen and Berrill have discarded the possible explanation of the behavior of the attached particles in their experiments on the basis of cortical movements. Such movements are common in eggs and may be easily identified by means of definitely localized vitally stained areas which may be followed through ensuing stages. This method was used by one of us (G. V.) at Roscoff [1] during the summer of 1935 with outstanding success. We shall mention here only the results necessary to dispel the doubt thrown by Cohen and Berrill on the significance of the polar "pit," perhaps better called the polar "spot." In addition, we shall describe briefly an anomaly observed by the senior author (A. D.) which gives further proof of the existence of a maturation spindle immediately under the polar spot.

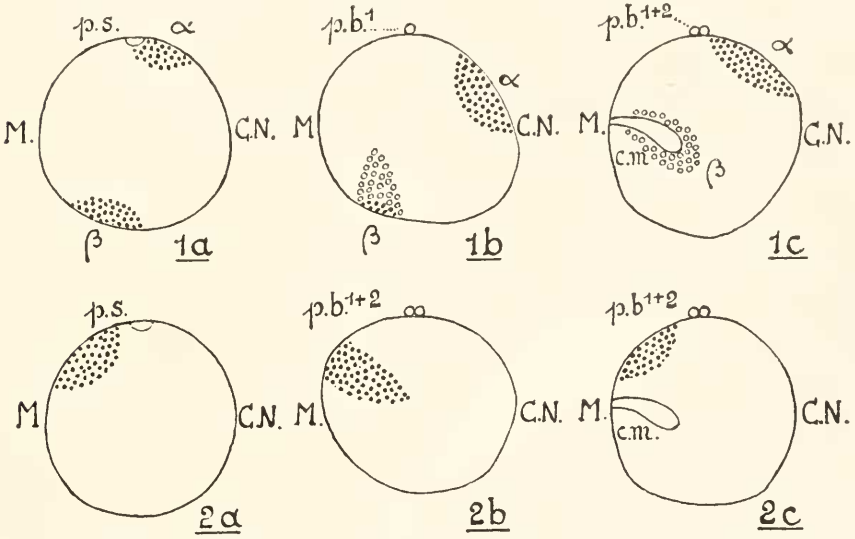
The ripe egg of *Ascidiella* [2] contains the first maturation spindle, as stated by Cohen and Berrill, and not the second, as previously stated by Dalcq on the basis of the examination of unfertilized eggs and the appearance of the spindle in sectioned preparations. On continuous observation two polar bodies may be seen to be successively extruded by the egg after fertilization. The jelly of the egg was removed with the aid of mounted needles (watchmakers' forceps can also be used), some dozens of naked eggs easily being prepared in but a few minutes. The method is not tedious.

In the discussion of merogony experiments Dalcq has already called attention to the fact that after insemination of naked eggs, the penetration of the spermatozoön into the egg may be delayed by as much as

[1] We wish to express our gratitude to Professor Dr. Ch. Pérez and Dr. G. Teissier for the excellent working opportunities accorded both of us during several stays at the "Laboratoire Lacaze-Duthiers."

[2] Because of some confusion as to the identification of the species used, it is necessary to state that our experiments were made on the *Ascidiella* which is found in abundance in the great pool of the Roscoff Laboratory, and which was used by Dalcq and by Tung in 1931 and 1932, and called by them *Ascidiella aspersa*. Berrill (1928) suggests that, because of the small size of the animal and the fact that the eggs do not float, the Roscoff species should be called *Ascidiella scabra*, the name of *Ascidiella aspersa* being reserved for the great *Ascidiella*, with floating eggs, common in Plymouth (and in the vicinity of Boulogne). Dalcq and Tung, following this suggestion, used the name *Ascidiella scabra* in their subsequent publications.

half an hour. Vandebroek has more recently discovered, in the course of experiments which will be reported in detail elsewhere, that the moment of sperm penetration can be recognized by a series of characteristic deformations of the egg which persist for about two minutes. The egg then returns to the spherical condition until the time of first polar body formation. As is noted below, the first and second polar bodies are formed 6 and 20 minutes respectively after the penetration of the spermatozoön. The actual times vary with temperature but the above are those observed in the present experiments. In dealing with eggs in



FIGS. 1 AND 2. Three views of eggs subjected to local vital staining: (*a*) ripe egg; (*b*) after the formation of the first polocyte; (*c*) during the formation of the yellow crescent and hyaline plasma zone. Colored territories stippled in deep black, when superficial invaginated parts (Figs. 1*b* and *c*) marked with ooo; *p.s.*: polar spot; pb^1: first polocyte; pb^{1+2}: the two polocytes; *M.*: mesoblastic side; *C.N.*: chordoneural side; *c.m.*: mesoblastic region (yellow crescent). The two eggs are viewed from their left sides.

the brief period preceding maturation it is especially important to recognize the delay mentioned above and to identify the moment of sperm penetration. This, unfortunately, Cohen and Berrill have failed to do.

In the experiments reported below, localized areas of denuded ripe unfertilized eggs were vitally stained with the dye 'Brillant Cresyl.' The stained areas have a diameter of from 50 to 75 μ (ca) and persist, perfectly localized, during the hours immediately following fertilization. If the egg is not fertilized the dye rapidly diffuses. Three experiments which demonstrate the significance of the polar spot will be described.

In the first case (Figs. 1*a*, 1*b*, and 1*c*) one mark (α) was made in the immediate vicinity of the polar spot and another (β) on the vegetative pole (Fig. 1*a*). In the second case one mark only was made, near the polar spot (Fig. 2*a*). In the third egg the mark was located a short distance from the pole, between the polar spot and the equator (Figs. 3*a* and 3*a'*). Tracing the fate of mark α of the first egg, we see that two minutes after fertilization the angular distance between the polar spot and the adjacent border of the colored region is increased. Comparing this with the similar behavior in the other eggs, it may be noted that all of the marked areas on the animal hemisphere have in-

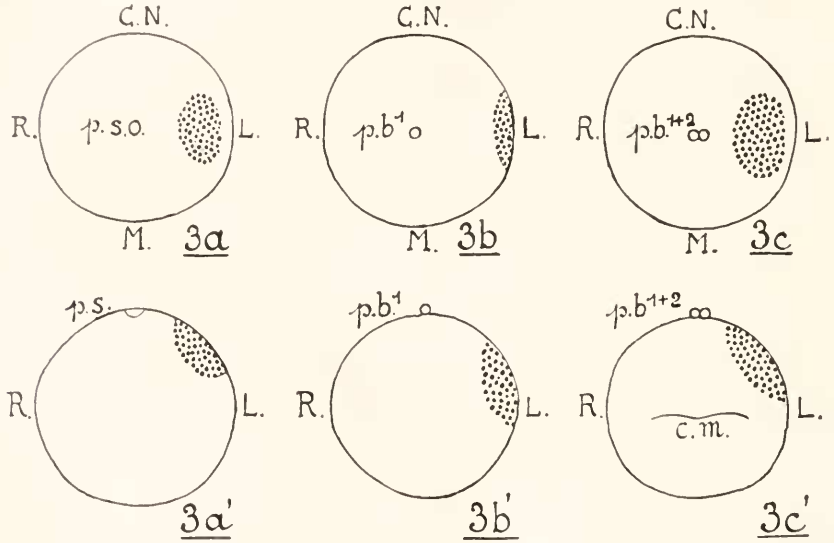


FIG. 3. An egg with lateral coloration of the animal pole. *a*, *b*, *c*: viewed from the animal pole; *a'*, *b'*, *c'*: viewed from the mesoblastic side. Other abbreviations as in Figs. 1 and 2.

creased their distance from the polar end by about 30° (Figs. 1*b*, 2*b*, 3*b*, and 3*b'*). They maintain these positions in spite of the considerable deformations which the egg undergoes during the period of expulsion of the two polar bodies. In the case illustrated by Fig. 1, the presence of the mark enables us to determine that the first polar body is actually formed in the polar spot about 6 minutes after fertilization. The second polar body is formed next to the first about 14 minutes later (Figs. 1*b* and 1*c*). It appears, therefore, that the polar spot is also the maturation site.

In the post-maturation phases during which the female pronucleus migrates toward the center of the egg to meet the male pronucleus and

the yellow crescent is gradually formed, the behavior of the marks in the vegetative pole is very interesting. This is to be described in detail elsewhere (by G. V.) but is suggested by the mark β of case 1. In the present consideration we are directly concerned with marks on the animal half of the egg and these consistently show a movement toward the animal pole which results in their return to their original positions (Figs. 1*c*, 2*c*, 3*c*, and 3*c'*). They show no further change during the cleavages which follow.

The appearance of the yellow crescent and the adjacent hyaline protoplasm which contains the fusion nucleus allows one to determine definitely the orientation of the egg. Using these landmarks, it was found that in the egg of Fig. 1, the mark α was in the plane of symmetry, just above the chordoneural material, while the mark β lay under the future yellow crescent, i.e. on the mesoblastic side of the germ. In the second egg the mark had been put between the polar spot and the presumptive mesoblastic crescent, nearly in the plane of symmetry. In the third case, on the other hand, the mark was located in a region to one side, in the left half of the germ. In these three typical experiments, therefore, the chief regions around the polar spot have also been explored.

To summarize briefly, it has been found that, after fertilization, the cortical layer of the ascidian egg undergoes definite displacements. During the initial period of active deformations, the cortical layer surrounding the animal pole moves toward the equator. Later, when the conjugation of the pronuclei takes place, the same material is again shifted nearer to the animal pole. In conclusion, it may be stated without the least hesitation that the polar spot indicates the site of formation of the polar bodies, and is the result of the presence of the first maturation spindle just below the cortex.

In view of these conclusions the question now arises as to how the data of Cohen and Berrill are to be interpreted. It seems clear that the variations they record in the distance between the adhering particle and the animal pole result from the activity of the cortical film; the cases in which the distance did not change may be explained on the assumption that the eggs were first observed somewhat too late. This explanation is not entirely satisfactory, however, for they appear to have observed some of the eggs continuously and yet fail to record any secondary shift of the materials towards the animal pole. In this connection we should like to make several suggestions inasmuch as it must be admitted that observations made by means of adhering particles are less reliable than those based on local vital staining of parts of the egg itself. The possibility of a displacement of the particle during the later deformations of

the egg should be examined. It is possible that the particle might eventually penetrate the cortical film and adhere directly to the underlying granular cytoplasm.

For the latter form, the morphological value of the polar spot is now established beyond any doubt and it would appear that Dalcq was justified in basing his merogony experiments on this indication of the animal-vegetative axis of the egg. Sectioning of the ripe ascidian egg in various planes relative to this primary axis and subsequent fertilization

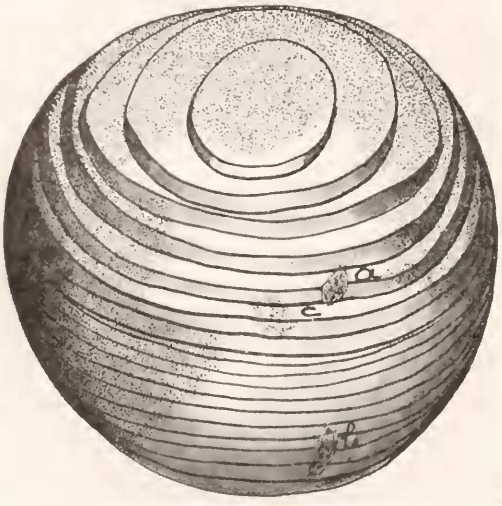


FIG. 4. Reconstruction of a fixed abnormal *Ascidiella* egg. Fixation, Allen; Sections, 6 μ; Staining, Hollande. Perspective view at 45° (method of Lison). *a, b, c:* the three maturation spindles. The eosinophil subcortical material is stippled.

of both pieces seems to be at present the sole method of analysing the distribution of the potencies at that stage. Further experiments have been carried on by the senior author during recent years and a short account of the results has been presented (Dalcq, 1935). This has led to a further cytological study of the structure of the ripe egg, and to the discovery of a preëxisting bilateral symmetry indicated by the arrangement of eosinophil subcortical granules (the material of the future yellow crescent) in the form of a large crescent which is more developed on one side of the primary axis. This important feature of the organization of the ripe *Ascidiella* egg together with the variations in the structure of merogonic twin embryos, whose anatomy has been thor-

oughly studied in a quantitative way, with several graphic reconstructions, will be fully described in another place.

In relation to the problem of the significance of the polar spot considered here, it seems important to draw attention to an anomaly which has to be borne in mind when performing merogony experiments. Among numerous sections of ripe eggs, the senior author has observed, four or five times, eggs which appear to possess two polar spots. Such eggs were at first discarded without further consideration, but later, a similar case was fixed and sectioned. The slide shows that the egg contains no less than three maturation spindles. In Fig. 4 may be seen a graphical reconstruction of the egg by Lison's method (1936). The three spindles lie in the region free from the eosinophil granules which nearly cover the vegetative hemisphere. The three spindles are small, of approximately equal size, and each supports some chromosomes. Owing to the direction of the plane of sectioning, which was more or less parallel to the primary axis of the egg, two of the spindles (*a* and *b*) lie under the part of the cortex toward the observer of the drawing; the third *c*, is situated on the opposite side, not so far from *b* as it appears to be on the drawing.

The infrequent occurrence of such eggs is of some importance to the investigator who is performing merogony experiments and throws some light on the significance of the polar spot. It is clear that the existence of a secondary spot corresponds here to a supplementary spindle. Why two and not three spots were seen may be explained in either of two ways; either one of the spots escaped the eye of the observer, or one of the spindles did not adhere sufficiently to the cortex to be seen *in vivo*. No positive information has been obtained concerning the origin of such anomalies. It seems probable that the spindle material became divided when the rupture of the germinal vesicle took place.

Summary

Ripe *Ascidiella* eggs have been subjected to local vital staining, chiefly in the regions surrounding the animal pole. Fertilization is immediately followed by a shift of the cortical layer towards the equator; when the yellow crescent appears, the material returns to its original position. The continuous observation of the ripe and the fertilized egg shows, contra Cohen and Berrill, that the polar spot marks the place where polocytes will be extruded.

In addition, attention is called to cases where there are two or three maturation spindles in the same egg.

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